Electroporation Protocol

Wash Competent Cells

1. Grow a liquid culture of DH5 alpha cells ~12 hours prior to the procedure.
2. Centrifuge the cells for 10 min at 4500rpms to create a pellet.
3. Decant the supernatant and resuspend in 2mL of chilled DepC-Treated Water.
4. Centrifuge the cells for 10 min at 4500rpms to create a pellet.
5. Decant the supernatant and resuspend in 1mL of chilled DepC-Treated Water
6. Centrifuge the cells for 10 min at 4500rpms to create a pellet.
7. Decant the supernatant and resuspend in 100µL of chilled 10% glycerol.
8. Centrifuge the cells for 10 min at 4500rpms to create a pellet.
9. Decant the supernatant and resuspend in 40µL of chilled 10% glycerol.
10. Keep the cells on ice until they are ready to be used.

Electroporation

Note: Prepare 5mL LB cultures and place them in the warm room, along with the appropriate number of plates 1hr prior to electroporation.

Note: Procedure should be done on ice until after the electroporation occurs.

1. Add 2-3µL of DNA to the competent cells, and transfer the mixture into an appropriately labeled chilled cuvette.
2. Remove the cuvette from the ice, and dry it thoroughly before placing it into the Electroporation machine.
3. On the Bacteria- EcoI setting, electroporate the cells.
4. Immediately after electroporating, resuspend the cells in 1mL of LB, then transfer all of the cells into warm 5mL cultures.
5. Repeat for all DNA samples.
6. Incubate the cultures at 37®C for 1hr.

Dilution

1. Plate 100µL of the cells onto warm agar plates with the correct antibiotic resistance.
2. If necessary, dilute the cells to grow single colonies.
3. Allow the plates to incubate at 37®C overnight.